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Antifungal Susceptibility Testing of *Candida* Isolates from the Candida Surveillance Study[▽]

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***Candida* species are a common cause of nosocomial bloodstream infections. Recent surveillance has shown an increase in the relative proportion of infections caused by *Candida glabrata*, which has reduced susceptibility to fluconazole. We undertook sentinel surveillance with antifungal susceptibility testing to monitor the trends in the proportions of various *Candida* species causing invasive disease. Forty-one institutions participated in the Candida Surveillance Study. All isolates were submitted to a central laboratory for identification and susceptibility testing. Susceptibility testing was performed in compliance with CLSI guidelines using a custom, broth dilution, microtiter system. There were 5,900 isolates submitted for identification and antifungal susceptibility testing. The distribution of species was as follows: *C. albicans*, 2,567 (43.5%) isolates; *C. glabrata*, 1,464 (24.8%) isolates; *C. parapsilosis*, 1,048 (17.8%) isolates; *C. tropicalis*, 527 (8.9%) isolates; *C. krusei*, 109 (1.9%) isolates; *C. lusitaniae*, 76 (1.3%) isolates; and other *Candida* species, 109 (1.9%) isolates. Resistance to fluconazole occurred in 1.2% of *C. albicans* isolates, 5.9% of *C. glabrata* isolates, 0.3% of *C. parapsilosis* isolates, and 0.4% of *C. tropicalis* isolates. Resistance to fluconazole was highly predictive of resistance to voriconazole. Resistance to echinocandins was rarely found, occurring in only 0.2% of all isolates. The rate of fluconazole susceptibility increased significantly from 87.5% in 2005 to 97.4% in 2007. The proportion of cases of disease caused by various *Candida* species did not change appreciably between 2004 and 2007, and the rate of antifungal susceptibility was high.**

Over the last 20 years, *Candida* species have become prominent nosocomial pathogens (1, 2, 21). Over the past decade, reports have documented a shift away from *Candida albicans* as the cause of the majority of invasive infections toward non-*C. albicans* species (7, 8, 19). *Candida glabrata*, which is less susceptible to fluconazole, is the species whose incidence has increased the most to account for the decrease in the proportion of cases of invasive disease caused by *C. albicans* (7, 8, 19, 21). The Centers for Disease Control and Prevention (CDC) conducted population-based surveillance for *Candida* bloodstream infections over two different time periods: in 1992 and 1993 and from 1998 to 2000 (7, 8). During the first surveillance period, *C. albicans* accounted for 52% of the isolates and *C. glabrata* accounted for 12%. During the second surveillance period, *C. albicans* accounted for 45% and *C. glabrata* accounted for 24%. During the latter surveillance period, when the activity of fluconazole was tested, the MIC_{50} and MIC_{90} were as follows: for *C. albicans*, ≤0.125 μg/ml and 0.5 μg/ml, respectively, and for *C. glabrata*, 4 μg/ml and 16 μg/ml, respectively (7, 8). In another sentinel surveillance study conducted from 1992 to 2001, the proportion of cases of disease caused by *C. glabrata* was only 18%, but the proportion did increase over the surveillance period in the United States (18). Contrary to the findings from the CDC surveillance, the other sentinel surveillance study found that the proportion of *C. glabrata* isolates which were susceptible to fluconazole increased from 15% in 1992 to 64% in 2001 (18). However, additional sentinel surveillance conducted by the same group between 1997 and 2005 did not show a significant shift in the proportion of cases of disease caused by *C. glabrata* (14). Additionally, that study did not detect any significant change in the rate of fluconazole resistance (14).

In order to monitor changing trends in the species distribution and antifungal susceptibility patterns of invasive *Candida* isolates, we undertook a sentinel surveillance program involving a variety of community and academic medical institutions in the United States.

MATERIALS AND METHODS

We collected isolates of *Candida* species from sterile body sites, e.g., blood, abscesses, joint fluid, and cerebrospinal fluid (CSF). Surveillance was conducted between September 2004 and December 2007. Forty-one institutions participated in the surveillance program. Most institutions were academic medical centers, but several were community or nonacademic medical centers. Each institution submitted between 25 and 200 consecutive isolates for identification and susceptibility testing. Only the initial isolate from each patient was submitted for evaluation.

All isolates were submitted to a central laboratory for identification and susceptibility testing. Identification of the isolates was done by using traditional microbiologic methods. The formation of germ tubes on incubation in serum was considered a definitive identification of *C. albicans*. No effort was made to differentiate *Candida dubliniensis* from *C. albicans*. A color change on CHROMagar medium (Sigma Aldrich) was used to identify mixed cultures and for the presumptive identification of *C. tropicalis*, *C. krusei*, and *C. albicans*. For the definitive identification of non-*C. albicans* species, we used the API 20C system (bioMerieux, Durham, NC) and the microscopic morphological appearance after growth on cornmeal-Tween agar. When traditional methods did not provide a conclusive species identification, sequencing of the rDNA was performed. Susceptibility testing was performed with a customized microtiter plate available from Trek Diagnostics (Cleveland, OH). Susceptibility testing was conducted according to the manufacturer's instructions, which comply with the Clinical and

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Laboratory Standards Institute (CLSI) guidelines outlined in document M27-A3 (3, 4). The agents included in the susceptibility testing plates included amphotericin B (0.06 to 8 μg/ml), fluconazole (0.12 to 256 μg/ml), voriconazole (0.008 to 16 μg/ml), posaconazole (0.03 to 64 μg/ml), caspofungin (0.008 to 16 μg/ml), and micafungin (0.008 to 16 μg/ml). For fluconazole and voriconazole, the MIC was determined by measurement of the concentration which resulted in a 50% reduction of growth after 24 h of incubation (3). For the echinocandins, the MIC was determined by detection of a significant reduction in growth after 24 h of incubation. For isolates which exhibited trailing, the MIC was read as the lowest concentration at which growth was reduced. However, reading of the susceptibility plates at 24 h greatly reduced the occurrence of trailing. Interpretation of the MIC (susceptible, susceptible dose dependent, and resistant) was performed in accordance with CLSI guidelines (4, 15–17); and the interpretations were as follows: for fluconazole, MICs of ≤8 μg/ml for susceptible, MICs of 16 to 32 μg/ml for susceptible dose dependent, and MICs of ≥64 μg/ml for resistant; for voriconazole, MICs of ≤1 μg/ml for susceptible, MICs of 2 μg/ml for susceptible dose dependent, and MICs of ≥4 μg/ml for resistant; and for caspofungin and micafungin, MICs of ≤2 μg/ml for susceptible and MICs of ≥4 μg/ml for resistant. For quality control, *C. krusei* and *C. parapsilosis* isolates from ATCC with defined susceptibility ranges were tested concurrently with the study isolates on a daily basis. The medical centers were designated either academic or community, as determined by the principle investigator at each site. Recently published data suggest that susceptibility breakpoints should be based on epidemiologic cutoff values (ECVs) (12). We used these recently published values, which are specific to the species, to determine additional rates of susceptibility and resistance to the echinocandins. These susceptibility cutoff values are as follows: for *C. albicans*, ≤0.12 μg/ml for caspofungin and ≤0.03 μg/ml for micafungin; for *C. glabrata*, ≤0.12 μg/ml for caspofungin and ≤0.03 μg/ml for micafungin; for *C. parapsilosis*, ≤1 μg/ml for caspofungin and ≤4 μg/ml for micafungin; for *C. tropicalis*, ≤0.12 μg/ml for caspofungin and ≤0.12 μg/ml for micafungin; for *C. krusei*, ≤0.24 μg/ml for caspofungin and ≤0.12 μg/ml for micafungin; and for *C. lusitaniae*, ≤0.5 μg/ml for caspofungin and ≤0.5 μg/ml micafungin (12).

Statistical analysis was done by using the SAS software package (release 8.02; Cary, NC). Where appropriate, a Cochran Mantel-Haenszel test or a Pearson correlation was used to test for significance.

RESULTS

Between September 2004 and December 2007, 5,900 yeast isolates were collected and submitted to the central laboratory for identification and susceptibility testing. The distribution of species was as follows: *C. albicans*, 2,567 (43.5%) isolates; *C. glabrata*, 1,464 (24.8%) isolates; *C. parapsilosis*, 1,048 (17.8%) isolates; *C. tropicalis*, 527 (8.9%) isolates; *C. krusei*, 109 (1.9%) isolates; *C. lusitaniae*, 76 (1.3%) isolates; *C. guilliermondii*, 14 (0.2%) isolates; *C. haemulonii*, 12 (0.2%) isolates; *C. keyfr*, 10 (0.2%) isolates; *C. lipolytica*, *C. pararugosa*, and *Trichosporon asahii*, 4 (0.1%) isolates each; *C. fermentati*, *C. rugosa*, *C. pelliculosa*, *Saccharomyces cerevisiae*, and *Lodderomyces elongisporus*, 3 (0.1%) isolates each; and *Pichia holstii*, *Pichia burtonii*, *Rhodotorula mucilaginosa*, *Geotrichum* species, *Saccharomyces elongisporus*, *Trichosporon* species, *Zygoascus* species, *C. bracarensis*, *C. catenulate*, *C. fabianii*, *C. inconspicua*, *C. intermedia*, *C. norvegensis*, *C. utilis*, and *C. zeylanoides*, 1 (0.04%) isolate each. Figure 1 shows the distribution of the six most common species.

Of the 5,900 isolates submitted for identification, 5,821 (98.5%) grew for susceptibility testing. The results of 24-h susceptibility testing for the six commonest species (*C. albicans*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, *C. krusei*, and *C. lusitaniae*) are presented in Table 1. Data are reported as the MIC ranges, the MIC_{50} and MIC_{90} values, the numbers of susceptible isolates, and the numbers of resistant isolates. Overall, fluconazole exhibited good activity against most species. In particular, *C. albicans*, *C. parapsilosis*, *C. tropicalis*, and *C. lusitaniae* were quite susceptible to fluconazole. In contrast,

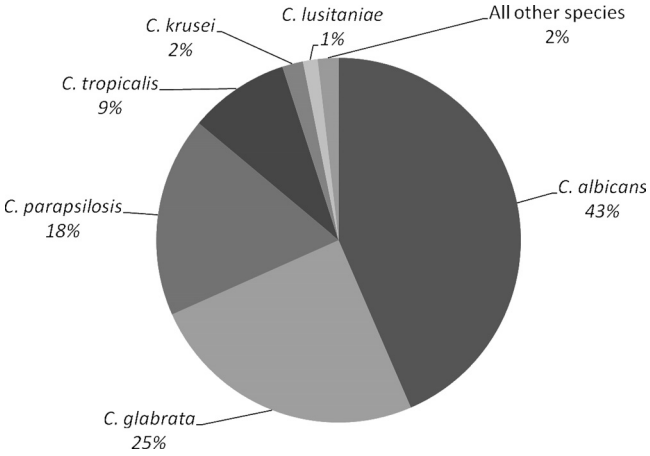

FIG. 1. Distribution of *Candida* species causing disease and isolated from sterile body sites during the Candida Surveillance Study, 2004 to 2007.

C. glabrata was less susceptible to fluconazole (MIC_{90}, 16 μg/ml). Both echinocandins, caspofungin and micafungin, exhibited excellent activity against all species of *Candida*, with the overall rate of susceptibility to both drugs being 99.8%. The rate of resistance to both drugs was low, at 0.2%. The echinocandins were quite potent against all species except *C. parapsilosis*, for which the MIC_{90} was 1 μg/ml. When the ECVs were applied to the echinocandins, the overall rates of resistance increased slightly. However, most isolates were slightly more resistant to caspofungin than to micafungin, but these differences were not statistically significant (Table 1).

Antifungal resistance. Overall, 133 (2.3%) isolates were resistant to fluconazole (MIC ≥ 64 μg/ml) (Table 2). The numbers of isolates of specific species resistant to fluconazole were as follows: *C. albicans*, 30 (1.2%) isolates; *C. glabrata*, 87 (5.9%) isolates; *C. parapsilosis*, 3 (0.3%) isolates; *C. tropicalis*, 2 (0.4%) isolates; and *C. lusitaniae*, 0 (0%) isolates. Resistance to fluconazole was highly predictive of voriconazole resistance (relative risk [RR] = 3.4, 95% confidence interval [CI] = 2.4 to 4.7, $R^2 = 0.83$, $P < 0.001$). Fluconazole resistance was not associated with echinocandin resistance (for caspofungin, RR = 0.97 and 95% CI = 0.94 to 1.00; for micafungin, RR = 0.97 and 95% CI = 0.94 to 1.00).

Trends over time. Thirteen centers contributed isolates during each of the years of surveillance. These centers contributed 3,068 (52%) of the isolates. To monitor for trends over time, we restricted our analysis to just those centers that contributed isolates in each of the 4 years. There was not a significant change in the distribution of species over time among the core centers (Table 3.) However, among these centers, there were significant differences in the rates of fluconazole susceptibility between 2004 and 2007. Between 2004 and 2005, the proportion of fully susceptible isolates decreased from 94.6% to 88.75%, and then the proportion steadily increased from 2005 to 2007, when the rate of susceptibility to fluconazole was 97.4% (Table 4). Similar trends were seen if the analysis was restricted to centers that contributed isolates only in 2005 to 2007, in which the surveillance continued throughout the year (data not shown). This trend was driven mainly by changes in

TABLE 1. *In vitro* activities of agents against 5,821 fungal isolates collected through the Candida Surveillance Study[a]

Species (no. of isolates)	Antifungal agent	MIC (μg/ml)			No. (%) of isolates		
		Range	50%	90%	Susceptible	Susceptible dose dependent	Resistant
All species (5,821)	Amphotericin B	≤0.12–≥8	1	1			
	Fluconazole	≤0.12–≥256	0.5	8	5,494 (94.5)	194 (3.3)	133 (2.3)
	Voriconazole	≤0.008–≥16	0.03	0.25	5,691 (97.8)	66 (1.1)	64 (1.1)
	Posaconazole	≤0.03–≥64	0.12	1			
	Caspofungin	≤0.008–≥16	0.06	0.5	5,807 (99.8)		14 (0.2)
	Micafungin	≤0.008–≥16	0.015	0.5	5,812 (99.8)		9 (0.15)
C. albicans (2,563)	Amphotericin B	≤0.12–≥8	1	1			
	Fluconazole	≤0.12–≥256	0.25	2	2,515 (98.1)	18 (9.3)	30 (1.2)
	Voriconazole	≤0.008–≥16	0.015	0.06	2,529 (98.7)	4 (0.2)	30 (1.2)
	Posaconazole	≤0.03–≥64	0.06	0.25			
	Caspofungin	≤0.008–≥16	0.03	0.12	2,558 (99.8)		5 (0.2)
	Micafungin	≤0.008–≥16	0.015	0.06	2,560 (99.9)		3 (0.1)
	Caspofungin ECV				2,487 (96.9)		80 (3.1)
	Micafungin ECV				2,500 (97.4)		67 (2.6)
C. glabrata (1,449)	Amphotericin B	0.5–≥8	1	1			
	Fluconazole	≤0.25–≥256	8	16	1,245 (86.1)	117 (8.0)	87 (5.9)
	Voriconazole	0.015–≥16	0.25	1	1,359 (93.9)	60 (4.1)	30 (2.1)
	Posaconazole	≤0.03–≥64	0.5	1			
	Caspofungin	≤0.008–8	0.06	0.12	1,445 (99.7)		4 (0.3)
	Micafungin	≤0.008–≥16	0.015	0.015	1,447 (99.9)		2 (0.1)
	Caspofungin ECV				1,330 (90.9)		134 (9.2)
	Micafungin ECV				1,428 (97.5)		36 (2.5)
C. parapsilosis (1,032)	Amphotericin B	0.25–4	1	1			
	Fluconazole	≤0.25–≥256	0.5	2	1,007 (97.6)	22 (2.1)	3 (0.3)
	Voriconazole	≤0.008–≥16	0.015	0.06	1,030 (99.8)	2 (0.2)	0 (0)
	Posaconazole	≤0.03–1	0.12	0.25			
	Caspofungin	≤0.008–2	0.25	1	1,031 (99.9)		1 (0.1)
	Micafungin	≤0.008–2	0.5	1	1,031 (99.9)		1 (0.1)
	Caspofungin ECV				1,030 (98.3)		18 (1.7)
	Micafungin ECV				1,048 (100)		0 (0)
C. tropicalis (523)	Amphotericin B	0.5–2	1	1			
	Fluconazole	≤0.12–≥256	0.5	2	518 (99.1)	3 (0.6)	2 (0.5)
	Voriconazole	≤0.008–≥16	0.03	0.12	520 (99.4)	0 (0)	3 (0.6)
	Posaconazole	≤0.03–≥64	0.12	0.25			
	Caspofungin	≤0.008–≥16	0.03	0.12	522 (99.8)		1 (0.2)
	Micafungin	≤0.008–4	0.015	0.03	522 (99.8)		1 (0.2)
	Caspofungin ECV				508 (96.4)		19 (3.6)
	Micafungin ECV				521 (98.9)		6 (1.1)
C. krusei (109)	Amphotericin B	0.5–2	1	2			
	Fluconazole	0.5–≥256	8	64	NA		NA
	Voriconazole	≤0.008–2	0.25	0.5	108 (99.1)	0 (0)	1 (0.9)
	Posaconazole	≤0.03–1	0.25	0.5			
	Caspofungin	≤0.008–0.5	0.12	0.25	108 (99.1)		1 (0.9)
	Micafungin	≤0.008–0.12	0.06	0.12	108 (99.1)		1 (0.9)
	Caspofungin ECV				103 (94.5)		6 (5.5)
	Micafungin ECV				108 (99.1)		1 (0.9)
C. lusitaniae (76)	Amphotericin B	0.5–2	1	1			
	Fluconazole	≤0.12–≥16	0.5	1	76 (100)	0 (0)	0 (0)
	Voriconazole	≤0.008–0.12	0.008	0.015	76 (100)	0 (0)	0 (0)
	Posaconazole	≤0.03–0.25	0.06	0.12			
	Caspofungin	0.03–1	0.25	0.5	76 (100)		0 (0)
	Micafungin	0.015–0.5	0.25	0.25	76 (100)		0 (0)
	Caspofungin ECV				74 (97.4)		2 (2.6)
	Micafungin ECV				76 (100)		0 (0)

[a] The breakpoints for susceptible, susceptible dose dependent, and resistant are those described in CLSI document M27-A3 (4). Additionally, for caspofungin and micafungin, the percentages of susceptible and resistant isolates are reported by using epidemiologic cutoff values (12).The breakpoints for susceptibility interpretation are as follows: for fluconazole, MICs of ≤8 μg/ml for susceptible, MICs of 16 to 32 μg/ml for susceptible dose dependent, and MICs of ≥64 μg/ml for resistant; for voriconazole, MIC of ≤ 1 μg/ml for susceptible, MICs of 2 μg/ml for susceptible dose dependent, and MICs of ≥4 μg/ml for resistant; and for caspofungin and micafungin, MICs of ≤2 μg/ml for susceptible and MICs of ≥4 μg/ml for resistant. NA, not applicable.

TABLE 2. *In vitro* antifungal susceptibility testing of fluconazole-resistant isolates

Species (no. of isolates)	Antifungal agent	MIC (μg/ml)			No. (%) of resistant isolates
		Range	50%	90%	
C. albicans (30)	Amphotericin B	0.5–≥8	1	2	
	Voriconazole	0.12–≥16	16	16	27 (90.0)
	Posaconazole	0.5–≥64	8	64	
	Caspofungin	≤0.008–≥16	0.03	2	3 (10.0)
	Micafungin	≤0.008–≥16	0.015	0.25	3 (10.0)
C. glabrata (87)	Amphotericin B	0.5–≥8	1	2	
	Voriconazole	0.5–≥16	2	4	29 (33.3)
	Posaconazole	0.5–≥64	2	4	
	Caspofungin	0.015–8	0.06	0.12	1 (1.1)
	Micafungin	≤0.008–≥16	0.015	0.03	1 (1.1)
C. parapsilosis (3)	Amphotericin B	1.0–1.0	1	1	
	Voriconazole	0.5–2	2	2	0 (0)
	Posaconazole	0.5–1	1	1	
	Caspofungin	0.5–1	0.5	1	0 (0)
	Micafungin	1–1	1	1	0 (0)
C. tropicalis (2)	Amphotericin B	1.0–1.0	1	1	
	Voriconazole	≥16–≥16	16	16	2 (100)
	Posaconazole	16–≥64	32	64	
	Caspofungin	0.03–0.06	0.03	0.06	0 (0)
	Micafungin	0.03–0.03	0.03	0.03	0 (0)

the proportions of susceptible *C. albicans* and *C. glabrata* isolates (Fig. 2).

Differences between academic and community medical centers. Table 5 shows the differences in the species distributions between the academic and the community medical centers. There was not a significant difference in the species distribution between the academic and the community medical centers. There were also no differences in the MIC_{50}s, MIC_{90}s, or the rates of occurrence of resistance to any antifungal agent between the academic and the community medical centers.

DISCUSSION

The data presented here demonstrate that non-*C. albicans* species continue to cause the majority of cases of invasive candidiasis. In our surveillance study, *C. albicans* was found to cause 44% of the cases of invasive disease. This is similar to the proportion of cases of disease caused by *C. albicans* that the CDC found in its latest population-based surveillance study conducted between 1998 and 2000 (7). However, the trend for an increasing proportion of disease to be caused by *C. glabrata* that was seen during the 1990s seems to have stabilized. Our surveillance study found that 25% of the isolates were *C. glabrata*. Again, this proportion is similar to what was found in the most recent CDC surveillance study (7). However, the proportion of cases of disease caused by *C. parapsilosis* was higher than that seen in the surveillance study of the CDC, and the proportion of *C. tropicalis* isolates causing invasive disease was lower. An increased rate of disease caused by *C. parapsilosis* has been noted previously and was related to echinocandin use. However, we did not collect data on echinocandin use by the participating centers and cannot confirm the earlier findings. The proportion of cases of disease caused by *C. krusei* was not dissimilar to that found by the CDC (7).

Overall, the *in vitro* susceptibility testing results were similar to those obtained in previous work (12–15). Fluconazole still tends to be quite active against most isolates of *Candida*. Pfaller et al. showed a relatively stable *C. albicans* MIC_{50} of 0.25 μg/ml over a 10-year period, between 1992 and 2001 (14, 18). Our MIC_{50} of 0.25 μg/ml is identical to that reported by Pfaller et al. (18). This indicates that there is not an ongoing decrease in the rate of fluconazole susceptibility, despite the continued widespread use of fluconazole both for therapy and

TABLE 3. Change in distribution of *Candida* species over time for the core institutions which contributed isolates throughout study period

Species	No. (%) of isolates			
	2004	2005	2006	2007
C. albicans	112 (40.4)	304 (43.9)	444 (42.6)	455 (43.1)
C. glabrata	68 (24.6)	186 (26.8)	232 (22.2)	260 (24.6)
C. parapsilosis	56 (20.2)	130 (18.8)	208 (19.9)	189 (17.9)
C. tropicalis	25 (9.0)	53 (7.7)	103 (9.9)	93 (8.8)
C. krusei	8 (2.9)	5 (0.7)	20 (1.9)	23 (2.2)
Others	8 (2.9)	15 (2.2)	36 (3.4)	35 (3.3)

TABLE 4. Changes in fluconazole susceptibility and resistance over the entire surveillance period[a]

Fluconazole susceptibility	No. (%) of isolates			
	2004	2005	2006	2007
Susceptible	262 (94.6)	615 (88.7)	1,000 (95.9)	1,027 (97.4)
SDD	10 (3.6)	44 (6.4)	28 (2.7)	20 (1.9)
Resistant	5 (1.8)	34 (4.9)	15 (1.4)	8 (0.8)

[a] Only data for isolates from centers contributing in all 4 years were included. There was a significant trend (χ^2, $P < 0.001$) toward decreasing fluconazole resistance. SDD, susceptible dose dependent.

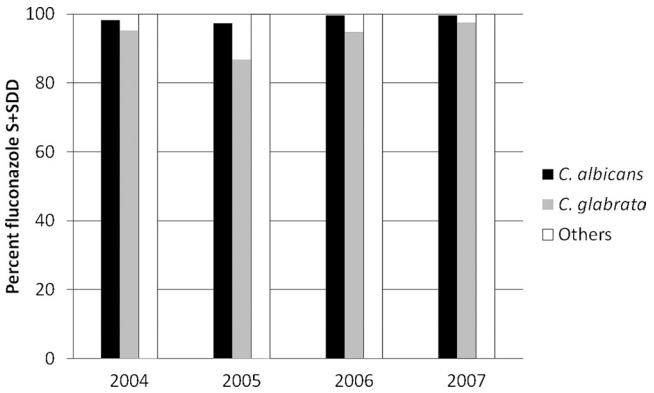

FIG. 2. Percentage of fluconazole-susceptible (S) and fluconazole-susceptible dose-dependent (SDD) *C. albicans*, *C. glabrata*, and other *Candida* sp. isolates over the 4 years of the study. After an initial drop in the number of susceptible isolates, there was a gradual increase between 2005 and 2007.

for prevention. It would appear that early concerns about the rapid development of resistance to fluconazole after its introduction are unfounded. To the contrary, we found that the rate of fluconazole resistance significantly declined over the last 3 years of surveillance.

We also found that resistance to fluconazole predicted resistance to voriconazole but not to caspofungin or micafungin, something noted by previous investigators (14, 16). The exception to this is for *C. krusei*, which is intrinsically resistant to fluconazole but against which voriconazole has excellent activity. This is not surprising and has been noted by other investigators (6). All azole antifungal medications have a common mechanism of action, i.e., inhibition of ergosterol synthesis (20). Thus, while specific mechanisms of resistance have not been described for the newer azoles (voriconazole and posaconazole), it is reasonable to assume that they are subject to the same mechanisms of resistance as the mechanisms of resistance to fluconazole. Those mechanisms of resistance include the upregulation of the CDR and MDR efflux pumps, as well as alterations in the gene for the target enzyme, ERG11 (20). However, echinocandins have a completely separate mechanism of action. They work by inhibiting 1,3-ß-D-glucan synthase (20). Thus, there is no reason to expect that resistance to fluconazole would also confer resistance to the echinocandins. Therefore, centers which test only for fluconazole sensitivity may wish to warn clinicians that voriconazole may have unreliable activity against fluconazole-resistant isolates.

The *in vitro* activities of both echinocandins were excellent against all species of *Candida* except *C. parapsilosis*. Even for *C. parapsilosis*, however, only 0.1% of the *C. parapsilosis* isolates were resistant to caspofungin or micafungin. The susceptibility data for the echinocandins and *C. parapsilosis* presented here agree with those from a recent clinical trial comparing caspofungin and micafungin (11). Both drugs successfully cleared most *C. parapsilosis* infections (11). However, in contrast to the findings presented in other reports, we did not find as great a disparity in susceptibility between the echinocandins caspofungin and micafungin (9). In 2003, Ostrosky-Zeichner et al. found, on average, that micafungin was 4 dilutions more potent than caspofungin, except when it was tested against *C. parapsilosis* (9). Except for testing against *C. parapsilosis*, we found that, on average, micafungin was only a dilution more potent than caspofungin. For *C. parapsilosis*, we found that the MIC_{90} of caspofungin was a dilution lower than that for micafungin. When we applied ECVs, as recommended by the European Committee on Antimicrobial Susceptibility Testing and described recently by Pfaller et al. (12), we found rates of resistance higher than those obtained by the use of standard CLSI breakpoints. In addition, our ECV-based rates of resistance were also slightly higher than those described by Pfaller et al. but are likely not statistically or clinically significantly different (12).

This study was limited in its ability to measure accurately the activity of amphotericin B. The broth microdilution method used here is not what the CLSI recommends for use for the determination of susceptibility to amphotericin B. We did not attempt to differentiate *C. dubliniensis* from *C. albicans*. However, *in vitro* the susceptibilities of *C. dubliniensis* are not different from those of *C. albicans* (5, 14). Because clinical outcomes data were not collected, we were also limited in our ability to relate *in vitro* resistance to poor clinical outcomes.

In summary, in this study of the *in vitro* antifungal susceptibilities of recently isolated *Candida* species, fluconazole was found to continue to be active against most isolates of *Candida*. However, less resistance to the echinocandins was detected, especially by *C. glabrata* and *C. krusei*, which have higher rates of resistance to fluconazole. Therefore, in institutions with high proportions of cases of invasive candidiasis caused by these two species, clinicians may wish to consider the use of a protocol in which echinocandins are used empirically, with or without susceptibility testing, until the species is known. Once the species is known, therapy can then be tapered to fluconazole, when appropriate, i.e., for the treatment of infections caused by *C. albicans*, *C. parapsilosis*, and *C. tropicalis*. These recommendations are in line with the guidelines of the Infectious Diseases Society of America on the management of candidiasis, which recommends the use of echinocandins as first-line therapy for moderate to severely ill patients (10). We also found that *in vitro* resistance to fluconazole was highly predic-

TABLE 5. Distribution of species among academic and community medical centers[a]

Medical center type	No. (%) of isolates					
	C. albicans	*C. glabrata*	*C. parapsilosis*	*C. tropicalis*	*C. krusei*	*C. lusitaniae*
Academic	2,125 (43)	1,216 (25)	882 (18)	447 (9)	95 (2)	61 (1)
Community	442 (45)	248 (25)	166 (17)	80 (8)	14 (1)	15 (2)

[a] There was no difference in the distribution of species on the basis of the designation of academic or community medical center. Chi-square general association, $P = 0.72$.

tive of resistance to voriconazole, which has implications for institutions which test only for fluconazole susceptibility and their choice for a second-line agent.

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